D-324 - Rpt #1(Final)
Contract: QMR&E (NATICK) #118
(Agreement)
Evaporated Milk Association



# Studies on Physical Stability and Tin Plate Deterioration in Cans of Evaporated Milk Held Under Refrigeration

Period: 27 April 1959 - 26 April 1961



QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES
Research and Engineering Command
Quartermaster Corps, U.S. Army
Chicago, Illinois

AD_____ Accession No._____

Evaporated Milk Association, Chicago, Ill.
Studies on Physical Stability and Tin Plate Deterioration in Cans of Evaporated Milk Held Under Refrigeration

Report No. 1(Final), Contract QMR&E (NATICK) #118 (Agreement)
Dr. E. H. Parfitt
Changes occurring in evaporated milk held for 4 years at refrigerated (32°F) temperature.

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CONTRACT RESEARCH PROJECT REPORT

QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES, CHICAGO
QM Research and Engineering Command, U. S. Army, QM Research and Engineering Center, Natick, Massachusetts

Evaporated Milk Association
228 North LaSalle Street
Chicago, Illinois

Official Investigator:
Dr. E. H. Parfitt

Project Nr. BP 2210.8
Contract: QMR&E (NATICK) #118
(Agreement)
Report Nr.: 1(Final)
File Nr.: D-324
Period: 27 April 1959 -
26 April 1961
Initiation Date: 27 April 1959

Title of Contract: Studies on Physical Stability and Tin Plate Deterioration in Cans of Evaporated Milk Held under Refrigeration

# A PHYSICAL AND CHEMICAL STUDY OF EVAPORATED MILK HELD FOUR YEARS AT 32° F.

Reported by the Administrative Technologists Committee
of the Evaporated Milk Association

## 1. Summary

Evaporated milk stored four years at 32°F. without turning or disturbing by moving has been analyzed for physical and chemical changes. Determination of iron and lead showed no significant difference from freshly produced evaporated milk packaged in the same type of container, but there appeared to be a pickup of tin over the storage period. This was also borne out in the examination of etching of the can. The vitamin content of $B_1$, $B_6$ and $B_{12}$ showed little loss, whereas $B_2$, riboflavin, was markedly reduced. Very little color change was noted on the stored samples as compared to a fresh control. Viscosity measurements and lime grain changed very little in the prolonged storage test. The pH of the stored samples was slightly reduced over that of the control. The protein availability held up surprisingly well, indicating the function of temperature is quite important, and the electrophoretic pattern was found to be essentially the same for all samples. Some differences were noted in the non-protein nitrogen and free fatty acids. Creaming and protein sedimentation was either not present or else present only in a minor degree.

## 2. Introduction

In May of 1955 a quantity of evaporated milk was purchased by the Quartermaster Corps and stored at 32° F. In the summer of 1959 representative samples from each lot were supplied the members of the Evaporated Milk Association, with the understanding that members of its Administrative Technologists Committee would subject this milk to physico-chemical analyses, the objective being to determine the degree of change that had taken place in the four-year storage period during which time the cans of evaporated milk were not turned or distrubed by moving. The balance of the evaporated milk was supplied to troops and was accepted without comment.

## 3. Experimental Procedure and Results

Eight different lots of evaporated milk stored without turning for four years at 32° F. were analyzed. The control used was a lot of evaporated milk made in 1959 at the same plant as that from which the stored milk was originally purchased; the control lot was milk produced at approximately the same time of year as the stored milk. Such a control sample was considered to be the best available.

### a. Iron, Tin, and Lead

The samples tested for iron were digested prior to analyses, using redistilled acid, and blank determinations were made on all reagents. The iron was determined colorimetrically by a thiocynate procedure using isoamyl alcohol to extract the color complex.

Tin and lead were determined on a Sargent Recording Polarograph (Model XXI). The tin and lead were removed from the acid digest by co-precipitation with added aluminum reagent.

Based on the average figures reported in Table I for iron, the arithmetic mean ($\bar{X}$) was found to be 1.8 ppm, and the standard deviation ($\sigma$) was $\pm$ 0.504 ppm. The coefficient of variation (Cv) was 28% which states that one deviation or 68% of the total concentration was 28% away from the average mean. Samples 7694, 7696, and 7699 were found to be significantly different from the average mean. The control sample averaged 1.9 ppm of iron. Based on the analysis of variance using Student's T, the average mean of the control and of the set of samples at the 1% level was not significant, nor at the 5% level.

Again, statistical analyses were made on averages of the samples for tin, with the arithmetic mean ($\bar{X}$) of 2.8 ppm and the standard deviation was $\pm$ 2.8 ppm. At the 5% level the difference between the control and the set of samples was found to be significant. Sample 7694 and 7695 were found to be quite a bit different from the average. This also borne out in the column on etching for the samples mentioned above.

The results for lead were found to be quite low for all samples including the control.

In Table 1 is reported the degree of etching inside the cans. The results in this column show a rough relationship to the amount of tin as shown in Column 3. Sample 7696 which had a high content of iron also revealed strong etching of the can.

b. Color

For color measurements a Beckman DU Model Spectrophotometer was used at two wave lengths, 520 millimicrons and 400 millimicrons, with the photo tube red and blue, respectively, and slit widths of 0.7 mm. and 0.025 mm.

The reflectance readings in Table 2 indicate that the individual stored samples had about the same color as the average of the group tested, with the average being 65.6% at 520 millimicrons as compared to the control of 68%. Approximately 1.5% difference in reflectance is distinguishable by the naked eye. Sample 7696 was slightly higher than average in color value at 520 millimicrons, and it will be recalled that iron was highest in this sample. Sample 7694 with the lowest reflectance at 520 millimicrons was highest with respect to tin content. At 400 millimicrons the average color was 46.5% against a control of 55.5%. Sample 7623 had a much lower color rating with this wave length. The analysis of variance indicated, however, that there was not enough difference among these samples to be of any significance.

c. Whipping

Whipping quality values were obtained as follows: Milk samples, mixing bowl and beaters were cooled to 35 to 42° F., 200 ml. of milk were poured into the cold mixing bowl and whipped for 2 minutes. The resulting product was poured into a 1,000 ml. graduated cylinder (not precooled) and the volume of whipped milk was read immediately and recorded as % over-run. The graduated cylinder was left to stand at room temperature. At intervals of 30, 60, and 90 minutes, the breakdown of the whip was observed by noting the amount of serum drainage. The data on whipping qualities are presented in Table 3.

d. Sensory Evaluation

A sensory evaluation was made wherein the four-year-old evaporated milk samples were checked as a beverage and also added to coffee. The results, using a hedonic rating, are given in Table 4. Based on these findings, there was no difference between the fresh and four-year-old samples when served in coffee, but a significant difference was found between the fresh and the stored evaporated milk when served as a beverage.

e. Creaming and Protein Sedimentation

The amount of creaming and protein sedimentation which occurred during this period was checked by visual observation. Data on observations are presented in Table 5. Based on these observations, it would appear there is no need for turning of evaporated milk even on extended storage if it is stored at a temperature of 32° F.

f. Viscosity, pH, and Lime Grain

The viscosity was measured by both a Mojonnier and a Brookfield viscosimeter at 75° F. The average Mojonnier units (Mu) for the four-year-old milk was 17 as compared to the control of 17. The Brookfield average was 28 centipoise as compared to 20 for the control. Sample 7694 was rather high in viscosity. The average Mojonnier units for the samples was 17 with a standard deviation of ± 4.8 Mu, indicating quite a variation among the samples tested.

The pH was measured with a Beckman Model G Glass Electrode; pH values of the stored samples were slightly lower than the control, but not significantly so.

Lime grain varied slightly for all the samples, but it was not enough to be of any significance.

The data on viscosity, pH, and lime grain measurements are presented in Table 6.

g. B Vitamins and Protein Quality

Samples were analyzed for $B_1$, $B_2$, $B_6$, and $B_{12}$, as well as to determine the biological availability of the protein. The results are given in Table 7 which also includes data on protein, fat and solids content of the samples, that is necessary to collect for use in the study of protein volume.

Riboflavin and thiamine were determined by fluorometric methods. Vitamin $B_6$ was determined microbiologically, using the yeast, S carlsbergensis, by comparison with fresh USP standard yeast cake. The USP method (Fourth Supplement, XIV, Revision 14, 1950) was used in determining vitamin $B_{12}$.

The data for vitamin $B_{12}$ content shows that none of the samples had as much $B_{12}$ as the control, but it is important to note that the average was 1.7 mmc g/g as compared with 2.5 mmc g/g for the control. After such a long time period in storage there was still evidence of vitamin $B_{12}$ present.

The study of protein quality was made with the Sprague-Dawley Weanling method on rats and was continued for six weeks. Seven groups of ten rats each were used; food was supplied ad lib, and daily records of food consumption were kept. The animals were weighed weekly, but only the final data are summarized here. Protein was supplied on a level of 9% on a dry basis. The rations were balanced between groups on a calorie basis, and they were adequately supplemented with thiamine, riboflavin, vitamin $B_6$, calcium and pantothenate, niacin, vitamin $B_{12}$, and minerals.

In 1956 in connection with another experiment, data was collected on the thiamine and riboflavin content of ten fresh evaporated milk samples. These data are shown for comparative pruposes in Tables 8 and 9. It is obvious that no measurable loss of thiamine has occurred durint the 32° storage period. Furthermore, an analysis of variance shows the difference between the 1956 milk data and the stored milk data is not significant statistically. The F value is 1.5, and 4.6 is required for significance at the 95% level.

In contrast, the riboflavin content of evaporated milk stored at 32° F. for four years is markedly lower than that of fresh samples. A comparison was made to the 1956 and later to the 1960 samples. An analysis of variance considering the storage sample data and the 1956 data gave an F value of 618.6, which is highly significant, since an F of only 8.53 is required for significance at the 99% level. Other studies have shown that riboflavin loss occurs during the storage in tinplate containers. The loss is minimized by storage in brown glass bottles or in lacquered cans because they eliminate iron and tin pickup. It is probable that the tin-iron-milk reaction contributes a reducing system which converts riboflavin to its less stable leuco form, which is lost. A rather strong reducing agent such as hydrosulfite or nascent hydrogen is required. Long storage in the reduced state probably results in the loss noted. The loss is believed to proceed more rapidly at higher storage temperatures.

Referring again to Table 7, no loss of vitamin $B_6$ is indicated, but it should be remembered the methods for this vitamin are less reliable than riboflavin or thiamine. No significant losses of protein quality are indicated by the data in Table 7. The rats fed the protein from a recently processed sample gained very slightly more than those fed the stored milk, but analysis of variance indicates the difference between groups is not significant, (F value only 0.38). When the group fed the recently processed samples is compared to all those fed the stored samples, the F value is also low, 1.16, again indicating no significance. When the comparison is made on the basis of grams gained per gram protein consumed, (P.E. protein efficiency), there is less difference between groups and the best value is given by one stored sample. An analysis of variance gives an F. value of 1.009 between all groups and an F value of 1.046 comparing the recently processed sample with all the stored samples. Moreover, on a percentage

basis, the latter difference amounts to only 2.4%. The protein quality in the samples stored at 32° has indeed been remarkably well perserved. This confirms and extends earlier observations that the effect of storage temperature on the loss of protein quality is minimal.

The results of analyses for protein nitrogen, free fatty acids and the electrophoretic pattern are shown in Table 10. Non-protein nitrogen values were obtained by modification of the Rowland method (J. Dairy Research 9, 42 (1938).) Free fatty acids were determined by the method described by Frankel and Tarassuk (J. Dairy Science 38, 751 (1955).) The electrophoretic mobility was determined in veronal pha. 5, using an Aminco Electrophoresis unit. The average time run was 10,000 seconds. Electrophoretic patterns were essentially the same for all samples, including the control. The average NPN was 0.86 as compared to 0.75% for the control sample. Free fatty acids had an average of 0.578 as compared to 0.38 on the control. Sample 7689 was rather high and 7696 was low in free fatty acids. Non-protein nitrogen was high in Sample 7623.

Acknowledgment

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Table 1

ANALYSES OF REFRIGERATED STORED QM EVAPORATED MILK SAMPLES

| Sample | IRON Parts per Million | TIN Parts per Million | LEAD (duplicate) Parts per Million | Etching |
|---|---|---|---|---|
| 7621 | 1.7 | 3.4 | Less than 0.1 | Moderate - uniform |
| 7622 | 1.7 | 1.4 | Less than 0.1 | Strong mod. - uniform |
| 7623 | 1.8 | 1.1 | Less than 0.1 | Moderate - uniform |
| 7689 | 1.8 | 1.3 | Less than 0.1 | Strong mod. - uniform |
| 7694 | 1.2 | 8.2 | Less than 0.1 | Strong mod. - uniform |
| 7695 | 2.1 | 0.6 | Less than 0.1 | Moderate - uniform |
| 7696 | 2.9 | 4.3 | Less than 0.1 | Strong mod. - uniform |
| 7699 | 1.2 | 2.0 | Less than 0.1 | Severe - uniform |
| Fresh Control | 1.9 | 1.5 | Less than 0.1 | Trace - uniform |

Table 2

PER CENT COLOR REFLECTANCE

| Photo-tube<br>Slit Opening<br>Wave Length | Red<br>.7 mm.<br>520 mu. | Blue<br>.025 mm<br>400 mu. |
|---|---|---|
| Lot No. 7621 | 65.0% | 45.5% |
| Lot No. 7622 | 65.5 | 46.5 |
| Lot No. 7623 | 66.8 | 43.5 |
| Lot No. 7689 | 66.0 | 49.0 |
| Lot No. 7694 | 64.5 | 46.3 |
| Lot No. 7695 | 65.0 | 47.2 |
| Lot No. 7696 | 66.0 | 47.5 |
| Lot No. 7699 | 66.5 | 46.5 |
| Fresh Control | 68.0 | 55.5 |

Table 3

WHIPPING QUALITY

| | Per Cent Overrun | % Serum Drainage | | |
|---|---|---|---|---|
| | | 30 Min. | 60 Min. | 90 Min. |
| Lot No. 7621 | 390% | 5% | 25% | 65% |
| Lot No. 7622 | 350 | 5 | 30 | 70 |
| Lot No. 7623 | 390 | 5 | 50 | 80 |
| Lot No. 7689 | 365 | 5 | 15 | 65 |
| Lot No. 7694 | 320 | 2 | 10 | 17 |
| Lot No. 7695 | 385 | 5 | 35 | 70 |
| Lot No. 7696 | 395 | 32 | 65 | 92 |
| Lot No. 7699 | 400 | 10 | 50 | 85 |
| Fresh Control | 370 | 10 | 45 | 65 |

Table 4

SENSORY EVALUATION (N-40)

| | As a Beverage | In Coffee | Combined |
|---|---|---|---|
| Four Year Old | 5.3 | 6.0 | 5.7 |
| Fresh Control | 6.1 | 5.8 | 6.0 |
| Average | 5.7 | 5.9 | 5.9 |

Table 5

VISUAL OBSERVATIONS ON CREAMING AND SEDIMENTATION

| Sample | Creaming | Protein Sedimentation |
|---|---|---|
| 7621 | None | Slight |
| 7622 | None | None |
| 7623 | Slight | Slight |
| 7689 | Slight + | Trace |
| 7694 | None | None |
| 7695 | None | Slight trace |
| 7696 | None | Slight trace |
| 7699 | None | Slight + |

NOTE: Samples were visually compared with a commercially produced evaporated milk which had been held for 2 years at room temperature. This sample used for comparison purposes showed a definite creaming.

Table 6

## EVALUATION OF FOUR YEAR OLD EVAPORATED MILK

| Code | | Viscosity M.U. | Viscosity c.p. | Temp. °F | pH | Lime Grain |
|---|---|---|---|---|---|---|
| 7621 | A | 15 | 23.1 | 74 | 6.19 | Trace |
| | B | 13 | 21.0 | 74 | 6.17 | Trace |
| | C | 14 | 21.5 | 74 | 6.19 | Trace |
| | Avg. | 14 | 21.9 | 74 | 6.18 | Trace |
| 7622 | A | 14 | 23.5 | 75 | 6.18 | Light |
| | B | 17 | 31.0 | 75 | 6.18 | Light |
| | C | 13 | 24.0 | 75 | 6.18 | Light |
| | Avg. | 14.7 | 26.2 | 75 | 6.18 | Light |
| 7623 | A | 16 | 25.0 | 75 | 6.17 | Light |
| | B | 16 | 25.6 | 75 | 6.17 | Trace |
| | C | 15 | 25.2 | 75 | 6.17 | Light |
| | Avg. | 15.7 | 25.2 | 75 | 6.17 | Light |
| 7689 | A | 15 | 21.5 | 74 | 6.18 | Trace |
| | B | 16 | 24.5 | 74 | 6.18 | Trace |
| | C | 15 | 23.5 | 74 | 6.19 | Trace |
| | Avg. | 15.3 | 23.2 | 74 | 6.18 | Trace |
| 7694 | A | 30 | 61.5 | 74 | 6.15 | Light |
| | B | 31 | 61.5 | 74 | 6.15 | Trace |
| | C | 25 | 45.5 | 74 | 6.16 | Med. |
| | Avg. | 28.3 | 56.2 | 74 | 6.15 | Light |
| 7695 | A | 20 | 31.0 | 73 | 6.12 | Lt. Med. |
| | B | 20 | 32.0 | 73 | 6.13 | Light |
| | C | 23 | 31.5 | 73 | 6.13 | Light |
| | Avg. | 21 | 31.5 | 73 | 6.13 | Light |
| 7696 | A | 13 | 21.0 | 75 | 6.20 | Light |
| | B | 14 | 23.5 | 75 | 6.21 | Light |
| | C | 14 | 22.4 | 75 | 6.19 | Light |
| | Avg. | 13.7 | 22.3 | 75 | 6.20 | Light |
| 7699 | A | 14 | 24.0 | 74 | 6.18 | Light |
| | B | 14 | 22.0 | 75 | 6.18 | Light |
| | C | 14 | 23.5 | 75 | 6.18 | Trace |
| | Avg. | 14 | 23.2 | 74.7 | 6.18 | Light |
| Fresh Control | A | 15 | 19.0 | 75 | 6.30 | None |
| | B | 17 | 20.4 | 75 | 6.29 | None |
| | C | 18 | 21.5 | 75 | 6.29 | None |
| | Avg. | 16.7 | 20.3 | 75 | 6.29 | None |

Table 7

DATA SUMMARY

VITAMIN CONTENT AND PROTEIN EFFICIENCY OF EVAPORATED MILK STORED FOUR YEARS AT 32° F.

| | Per Cent | | | Mg. per Liter of Evaporated Milk | | | Protein Quality | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | Protein | Fat | Solids | Thiamine | Riboflavin | Vitamin $B_6$ | Average 6 Weeks Rat Gain in Grams | Average Grams Gain / Grams Protein | Vit. $B_{12}$ mmcg/g |
| 7621 | 7.50 | 7.93 | 26.65 | 0.54 | 3.20 | 0.55 | 114.7 | 2.84 | 1.8 |
| 7622 | -- | 7.93 | 26.30 | 0.55 | 2.99 | 0.52 | -- | -- | 1.7 |
| 7623 | 7.30 | 7.91 | 26.21 | 0.51 | 3.08 | 0.42 | 111.4 | 2.92 | 1.6 |
| 7689 | -- | 7.92 | 26.63 | 0.55 | 3.21 | 0.43 | -- | -- | 1.6 |
| 7694 | 7.57 | 7.90 | 26.81 | 0.60 | 2.91 | 0.51 | 108.3 | 2.75 | 1.9 |
| 7695 | 7.44 | 7.89 | 26.36 | 0.51 | 2.82 | 0.49 | 110.1 | 2.83 | 2.1 |
| 7696 | 7.32 | 7.92 | 26.10 | 0.62 | 2.90 | 0.42 | 112.8 | 2.84 | 2.0 |
| 7699 | 7.31 | 7.91 | 26.26 | 0.58 | 2.86 | 0.43 | 110.3 | 2.85 | 1.9 |
| Average | 7.41 | 7.91 | 26.41 | 0.56 | 2.99 | 0.47 | 111.3 | 2.84 | |
| Fresh Control | | | | | | | | | 2.5 |
| G 23 | 7.22 | 7.95 | 26.11 | Fresh | 0.53 | 3.84 | 0.47 | 116.8 | 2.91 | |
| G 2 | -- | -- | -- | Production | 0.62 | 3.84 | 0.42 | -- | -- | |
| G 7 | -- | -- | -- | Same Source | 0.61 | 3.74 | -- | -- | -- | |

Table 8

THIAMINE
Mg. per Liter

| Samples Stored at 32° F. for Four Years | Galt, Calif. Nov.-Dec. 1956 Samples Analyzed Soon After Processing |
|---|---|
| 0.54 | 0.64 |
| 0.55 | 0.53 |
| 0.51 | 0.56 |
| 0.55 | 0.58 |
| 0.60 | 0.46 |
| 0.51 | 0.52 |
| 0.62 | 0.47 |
| 0.50 | 0.54 |
| | 0.56 |
| | 0.56 |
| Average 0.56 | 0.54 |

Table 9
RIBOFLAVIN
Mg. per Liter

| | | Galt, Calif. Samples Analyzed Soon After Processing | |
|---|---|---|---|
| | Samples Stored at 32° F. for Four Years | Nov.-Dec. 1956 Samples | Jan. 1960 Samples |
| | 3.20 | 3.94 | 3.84 |
| | 2.99 | 3.70 | 3.74 |
| | 3.08 | 3.88 | 3.74 |
| | 3.21 | 3.78 | 3.84 |
| | 2.91 | 3.82 | 3.98 |
| | 2.82 | 3.68 | 3.84 |
| | 2.90 | 4.12 | 3.80 |
| | 2.86 | 4.16 | 3.91 |
| | | 3.94 | |
| | | 4.04 | |
| Average | 2.99 | 3.91 | 3.84 |

Table 10

| Lot | % Non-Protein Nitrogen | Fatty Free Acids | Electrophoretic Mobility $cm^2$ volts $^{-1}$ $sec^{-1}$ descending components I | descending components II | ascending components I | ascending components II |
|---|---|---|---|---|---|---|
| 7621 | .83 | .444 | 5.1 | 2.6 | 7.8 | 5.1 |
| 7622 | .82 | .512 | 5.1 | 2.6 | 7.8 | 5.6 |
| 7623 | 1.07 | .568 | 5.0 | 2.5 | 7.6 | 4.5 |
| 7689 | .81 | 1.052 | 4.7 | 2.3 | 7.9 | 4.9 |
| 7694 | .84 | .580 | 5.0 | 3.4 | 7.5 | 5.3 |
| 7695 | .81 | .500 | 4.7 | 2.9 | 7.5 | 5.3 |
| 7696 | .82 | .396 | 4.9 | 2.9 | 7.9 | 5.0 |
| Fresh Control | .74 | .380 | $5.4 \times 10^{-5}$ | $2.7 \times 10^{-5}$ | $6.9 \times 10^{-5}$ | $4.4 \times 10^{-5}$ |
| Avg. | .86 | .578 | | | | |